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Synthesis and antimicrobial activity of piperidin-4-one derivatives

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ABSTRACT

The present work to investigate the newer antibacterial and antifungal Agents with less side effect and potent activity.In the present work, a series of 2,6-diaryl-3-methyl-4-piperidones were synthesized by Mannich reaction (condensation) of ethyl methyl ketone, benzaldehyde, substituted aromatic aldehydes and ammonium acetate. Thiosemicarbazone derivatives of 2,6-diaryl-3-methyl-4-piperidones were synthesized by reaction of 2,6-diaryl-3-methyl-4-piperidones with thiosemicarbazide. All the title compounds have been screened for their in vitro antibacterial activity against various strains. Some of these title compounds exhibited significant antimicrobial activity (compared with ampicillin) and antifungal activity (compared with terbinafine). The present study reveals that these compounds could be used as a template for the future development through modification or derivatization to design more potent antimicrobial agents.

Key words: Piperidin-4-one, Thiosemicarbazone, Antibacterial agents, Antifungal agents.

INTRODUCTION

Infection is a major category of human disease and skilled management of antimicrobial drugs is of first importance. The spread of antimicrobial resistance among pathogenic bacteria has become a serious problem for the clinical management of infectious diseases and has resulted in a clear need for novel antimicrobial agents other than analogs of existing antibiotics[1]. Among the wide variety of heterocycles that have been explored for developing pharmaceutically important molecules, Piperidin-4-ones exhibit various biological activities like analgesic, hypotensive and central nervous system depressant, antiviral, bactericidal and fungicidal activities[2,3,4,5]. The present work was undertaken with a view to explore the possibility of antibacterial and antifungal activities in a piperidine ring having a thiosemicarbazone moiety.

Syntheses of title compounds were affected as outlined in the Scheme I. Ammonium acetate, benzaldehyde, 4-alkylbenzaldehyde and 2-butanone in ethanol were condensed to form 3-alkyl-2-(4'-aryl)-6-phenylpiperidin-4-ones (1a-6a)[6,7]. The respective ketone thiosemicarbazone (1b-6b) were prepared in good yields by condensing thiosemicarbazides with appropriate ketones, in the presence of trace amounts of conc. HCl. These were characterized by physical properties, IR and ^{1H}NMR spectral studies. The antimicrobial activities of the compounds were carried out according to the standard procedures.

MATERIAL AND METHODS

Ammonium acetate, benzaldehyde, 4-alkyl benzaldehyde and ethanol were obtained from SD Fine Chemicals Pvt. Ltd., Boisar. Thiosemicarbazide were obtained from E. Merck Ltd., Mumbai.

The melting points were carried out in an open capillary tube and were uncorrected. Thin layer chromatography was performed using silica gel coated on a glass plate and spots were visualized by exposure to iodine vapor. IR spectra in Nujol were recorded on a Shimadzu IR spectrophotometer. ^{1}H NMR spectra were recorded in DMSO on an av500 spectrometer using TMS as an internal standard (chemical shifts in δ ppm).

Synthesis of 3-alkyl-2-(4'-aryl)-6-phenylpiperidin-4-ones (1a-6a)

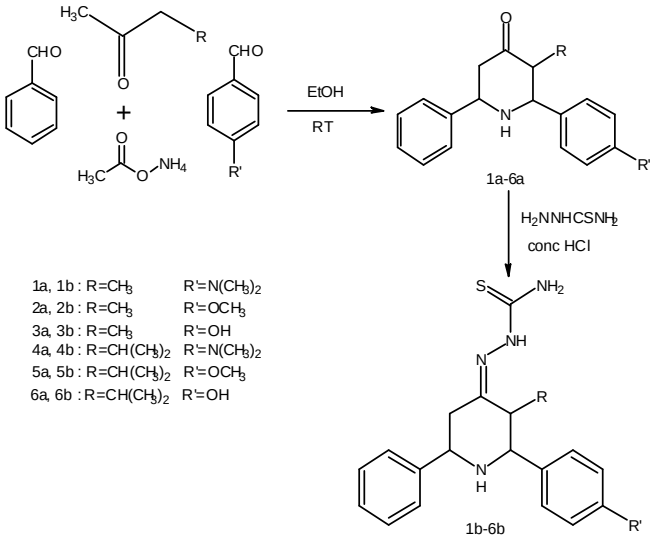
Dry ammonium acetate (0.1 moles) was dissolved in 50 ml ethanol and the solution was mixed with 4-alkyl benzaldehyde (0.1 moles), benzaldehyde (0.1 moles) and butane-2-one (0.1 moles). The mixture was heated to boiling and allowed to stand at room temperature overnight. Then 30 ml conc. HCl was added and the precipitated hydrochloride salt was collected, washed with mixture of ethanol and ether (1: 5). A suspension of the hydrochloride salt in acetone was treated with strong liquid ammonia and the free base was obtained by separating water. The crude product was recrystallised from ethanol to get the compound and thin layer chromatography was eluted in methanol: ethyl acetate.

2-[4-(dimethylamino) phenyl]-3-methyl-6-phenylpiperidin-4-one (Compound 1a): Yield-82.38%, m.p.– 188-190°C, Rf- 0.60, IR (KBr): 3426, 3039, 2936, 1722, 1459, 749 cm^{-1}, ^{1}H-NMR (DMSO-d_6): δ 7.4-7.7 (9H), 4.3-4.9(1H), 0.95(3H), 2.3-2.6(6H).

2-(4-methoxyphenyl)-3-methyl-6-phenylpiperidin-4-one (Compound 2a): Yield-80.33%, m.p.–170-172°C, Rf- 0.76, IR (KBr): 3230,2876,1650,1210 cm^{-1}.

2-(4-hydroxyphenyl)-3-methyl-6-phenylpiperidin-4-one (Compound 3a): Yield-69.39%, m.p.–154-156°C, Rf- 0.87, IR (KBr): 3256, 2976, 1719, 1457,1219 cm^{-1}, ^{1}H-NMR (DMSO-d_6): δ 7.4-7.7 (5H), 6.8-6.9(4H), 4.8(1H), 2.8(2H), 2.0-2.1(1H).

2-[4-(dimethylamino)phenyl]-3-isopropyl-6-phenylpiperidin-4-one (Compound 4a): Yield-72.32%, m.p.–196-198^{0}C, Rf- 0.57, IR (KBr): 3200, 2957,1702, 1458,1492 cm^{-1}, ^{1}H-NMR (DMSO-d_6): δ 7.2-7.5 (9H), 4.1(1H), 2.7-2.8(2H), 2.4-2.5(6H), 0.8-1.0(6H).



Scheme 1: Thiosemicarbazone derivatives of 2,6-diaryl-3-methyl-4-piperidones

Table 1: Spectral Data of Compounds 1a to 6b

Compound	IR (cm^{-1})	^{1}HNMR (d ppm)
1a	3426, 3039,2936,1722, 1459,749	7.4-7.7 (9H), 4.3-4.9(1H), 0.95(3H), 2.3-2.6(6H)
2a	3230,2876,1650,1210	-
3a	3256, 2976, 1719, 1457, 1401, 1219	7.4-7.7 (5H), 6.8-6.9(4H), 4.8(1H), 2.8(2H), 2.0-2.1(1H)
4a	3200, 2957,1702, 1458, 1492	7.2-7.5 (9H), 4.1(1H), 2.7-2.8(2H), 2.4-2.5(6H), 0.8-1.0(6H)
5a	3134, 1631, 1403,699	7.0-7.7 (9H), 4.6-4.8(1H), 3.8(3H), 2.9(1H), 1.0-1.1(6H)
6a		-
1b	3234,1756,1250	-
2b	3424, 3101, 2954, 1207-1082, 1627	7.0-7.4(9H), 4.5-4.7(1H), 3.8-
3b	3430, 3168, 1630,691	3.3(3H), 1.5-0.8(3H)
4b	3150, 2926, 1178-1063, 1457, 1226, 697	-
5b	3423, 2927-2812, 1149-1122, 1459,649	7.5(5H), 6.3-6.5(4H), 2.0-2.1(1H)
6b	3150, 2953, 1452,1210-1150 3256, 2976, 1457, 1219,657	-

Table 2: Antibacterial activity: MIC values (µg/mL) of compounds (1a-6a)

Compd	R	R'	*S. aureus* ATCC 6538	*E. coli* ATCC 8739	*B. subtilis* MTCC 441
1a	CH_3	$N(CH_3)_2$	12	8	10
2a	CH_3	OCH_3	12	6	15
3a	CH_3	OH	17	4	13
4a	$CH(CH_3)_2$	$N(CH_3)_2$	10	4	13
5a	$CH(CH_3)_2$	OCH_3	10	5	17
6a	$CH(CH_3)_2$	OH	16	6	15
Ampicillin			22	10	23

Table 3: Antibacterial activity: MIC values (µg/mL) of compounds (1a-6e)

Compd	R	R'	*S. aureus* ATCC 6538	*E. coli* ATCC 8739	*B. subtilis* MTCC 441
1b	CH_3	$N(CH_3)_2$	8	8	14
2b	CH_3	OCH_3	11	6	13
3b	CH_3	OH	15	4	14
4b	$CH(CH_3)_2$	$N(CH_3)_2$	10	3	14
5b	$CH(CH_3)_2$	OCH_3	12	6	13
6b	$CH(CH_3)_2$	OH	15	5	13
Ampicillin			22	10	23

3-isopropyl-2-(4-methoxyphenyl)-6-phenylpiperidin-4-one (Compound 5a): Yield-59.44%, m.p.–184-186^{0}C, Rf- 0.67, IR (KBr): 3134, 1631, 1403,699 cm^{-1}, ^{1}H-NMR (DMSO-d_6): δ 7.0-7.7 (9H), 4.6-4.8(1H), 3.8(3H), 2.9(1H),1.0-1.1(6H). 2-(4-hydroxyphenyl)-3-isopropyl-6-phenylpiperidin-4-one (Compound 6a): Yield-63.43%, m.p.–160-162°C, Rf- 0.83, IR (KBr): 3234, 1756, 1250 cm^{-1}.

Synthesis of 3-alkyl-2-(4'-aryl)-6-phenyl piperidin-4-thiosemicarbazones (1b-6b)

To a boiling solution of compounds 1a-6a (0.01 moles) in 45 ml methanol, added a few drops of conc. HCl. Thereafter thiosemicarbazide (previously dissolved in 20 ml methanol) solution (0.01 moles) was added dropwise with stirring. The reaction mixture was refluxed for 3 hour on a heating mentle. After cooling, the solid product was filtered off and recrystalised from 20 ml methanol to get compound and thin layer chromatography was eluted in methanol: ethyl acetate.

2-[4-(dimethylamino) phenyl]-3-methyl-6-phenylpiperidin-4-thiosemicarbazone (Compound 1b): Yield-68.26%, m.p. -230-232°C, Rf- 0.38, IR (KBr): 3424, 3101, 2954, 1207-1082,1627 cm^{-1}.

2-(4-methoxyphenyl)-3-methyl-6-phenylpiperidin-4- thiosemicarbazone (Compound 2b): Yield-59.78%, m.p.–222-224°C, Rf- 0.93, IR (KBr): 3430, 3168, 1630, 691cm^{-1}, ^{1}H-NMR (DMSO-d_6): δ 7.0-7.4(9H), 4.5-4.7(1H), 3.8-3.3(3H), 1.5-0.8(3H).

2-(4-hydroxyphenyl)-3-methyl-6-phenylpiperidin-4-thiosemicarbazone (Compound 3b): Yield-48.02%, m.p.–218-220°C, Rf- 0.65, IR (KBr): 3150,2926, 1178-1063, 1457,1226,697 cm^{-1}.

2-[4-(dimethylamino)-phenyl]-3-isopropyl-6-phenylpiperidin-4-thiosemicarbazone (Compound 4b): Yield-39.11%, m.p.–248-250^{0}C, Rf-0.35, IR (KBr): 3423, 2927-2812, 1149-1122, 1459, 649 cm^{-1}.

3-isopropyl-2-(4-methoxyphenyl)-6-phenylpiperidin-4-thiosemicarbazone (Compound 5b): Yield-45.22%, m.p.–238-240°C, Rf- 0.38, IR (KBr): 3150, 2953, 1452, 1210-1150 cm^{-1}, ^{1}H-NMR (DMSO-d_6): δ 7.5(5H), 6.3-6.5(4H), 2.0-2.1(1H).

2-(4-hydroxyphenyl)-3-isopropyl-6-phenylpiperidin-4-thiosemicarbazone (Compound 6b): Yield-39.26%, m.p.–228-230°C, Rf- 0.42, IR (KBr): 3256, 2976, 1457, 1219,657 cm^{-1}.

Antibacterial activity

The synthesized derivatives 1a-6b were screened for their *in vitro* antibacterial activity against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), *Bacillus subtilis* (MTCC-441), using disk diffusion method. Mueller-Hinton agar (Difco, Detroit, USA) was used for the bacterial strains. All compounds have shown good activity. MIC values of the compounds are given in Table 2 and 3.

Table 4: Antifungal activity: MIC values (µg/mL) of compounds (1a-6a)

Compd	R	R'	*M.gypseum* NCPF-580	*M.canis*	*T. megentag -rophytes*	*T. rubrum*	*C. albicans* ATCC 10231
1a	CH_3	$N(CH_3)_2$	7	7	8	8	14
2a	CH_3	OCH_3	-	-	-	-	-
3a	CH_3	OH	-	-	-	-	-
4a	$CH(CH_3)_2$	$N(CH_3)_2$	4	4	4	4	10
5a	$CH(CH_3)_2$	OCH_3	6	6	6	6	6
6a	$CH(CH_3)_2$	OH	-	-	-	-	15
Terbinafine			$\leq$ 0.25	$\leq$ 0.25	$\leq$ 0.25	$\leq$ 0.25	1

Antifungal activity

Study design

Micro dilution was used according to a standard protocol described by the NCCLS[10, 11]. Five strains were tested each of the following species: *Microsporum gypseum* NCPF-580, *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Candida albicans* ATCC 10231.

Medium

RPMI 1640 broth with L-glutamine without sodium bicarbonate and 0.165 µMOPS buffer (34.54 g/L) was used. The medium was adjusted to pH 7.0 at 25 ^{0}C. Sterility control of each bottle was performed before it was used.

Antifungal agents

Terbinafine was provided by the manufacturer as a standard powder. All drugs were dissolved in 100% dimethyl sulfoxide according to the NCCLS methods[10, 11]. The final drug concentrations were 32 to 0.01g/mL for all drugs.

Preparation of inoculum

The preparation of inoculum suspensions was based mainly on the NCCLS guidelines[21] and as described previously[12-14]. For dermatophytes the final inoculum size was adjusted from 1.2 X10^4 to 6 X10^4 CFU/mL and for *C. albicans* it was approximately 1 X 10^3 to 5 X 10^3 CFU/mL[10, 15, 16].

Table 5: MIC values (µg/mL) of compounds (1a-6b)

Compd	R	R'	*M.gypseum* NCPF-580	*M.canis*	*T. megentag -rophytes*	*T. rubrum*	*C. albicans* ATCC 10231
1a	CH_3	$N(CH_3)_2$	3	5	5	5	5
2a	CH_3	OCH_3	4	5	5	2	5
3a	CH_3	OH	3	3	3	3	5
4a	$CH(CH_3)_2$	$N(CH_3)_2$	3	3	3	3	8
5a	$CH(CH_3)_2$	OCH_3	4	5	3	2	8
6a	$CH(CH_3)_2$	OH	4	4	4	4	5
Terbinafine			$\leq$ 0.25	$\leq$ 0.25	$\leq$ 0.25	$\leq$ 0.25	1

Test procedure

The test procedure was applied according to the NCCLS protocols[10, 11]. Microdilution plates (96 U-shaped) were prepared and frozen at -70 ^{0}C until needed. Each microdilution well containing 100 µL of the 2-fold drug concentration was inoculated with 100 µL of the final inoculum suspension. Two drug-free growth controls were included for each test plate, one without any drug (growth control) and the other with media containing an equivalent amount of solvent used to dissolve the drug (solvent control). For all drugs, the minimum inhibitory concentration (MIC) was defined as the lowest concentration showing 100% growth inhibition. All of the compounds (1a-6b) were found to have antifungal activity against *M. gypseum*, *M. canis*, *T. mentagrophytes*, *T. rubrum* and *C. albicans*. MIC values of the compounds are given in Table 4 - 5.

RESULTS

All the synthesized compounds were confirmed by spectral data and then screened for antibacterial activity against *Staphylococcus aureus* (ATCC 6538), *E. coli* (ATCC 8739) and *Bacillus subtilis* (MTCC441) and for antifungal activity against *M. gypseum* (NCPF-580), *M. canis, T. megenagrophytes, T. rubrum* and *C.albicans* (ATCC 10231). All compounds have shown good activity when compared with standard drug ampicillin.

Compounds 2a, 3a and 6a has no antifungal activity while (1b-6b) have shown the significant antifungal activity when compared with standard drug terbinafine. The *in vitro* antibacterial and antifungal activities are presented in Table 2, 3, 4 and 5.

DISCUSSIONS

The antibacterial activity of all the synthesized compounds are good enough in comparison to ampicillin while the antifungal activity of thiosemicarbazone derivatives of piperidin-4-one are very high from piperidine-4-one which indicate the activity is enhanced by addition of this group.

CONCLUSION

The present study reveals that these compounds could be used as a template for the future development through modification or derivatization to design more potent antimicrobial and antifungal agents with fewer side effects.

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